College of Biological Sciences

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# Identifying key residues in the active site of 2SL (2-succino lyase)

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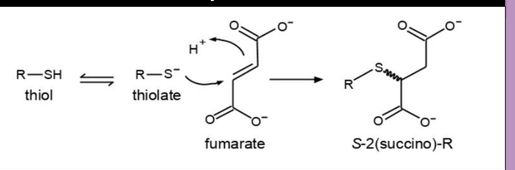
## BACKGROUND

1. Succination is a contributor to pathogenesis in diabetes, obesity, and certain cancers [10-13]




2. Succination forms the oncometabolite S-(2-succino)cysteine lyase (2SC)[1,2]

3. A breakdown pathway for 2SC is encoded by the *yxe* operon (Fig. 1). In *Enterococcus italicus* and *Dickeya dadantii* this operon includes the enzyme 2SL, a protein that acts on 2SNAC.[15]

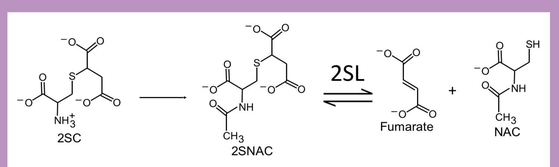
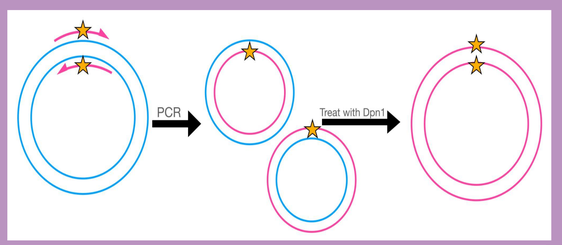



**Figure 1: 2SL breakdown reaction.** 2SL acts on 2SNAC to produce fumarate and NAC. This reaction is reversible.

## METHODS

**4. 3D Modeling 2SL (Fig. 2)**
**(i)** We created a 3D model of 2SL and its four active sites (using the the known crystal structure of Fumarate lyase from *Mesorhizobium*)
**(ii)** By comparing 2SL to related enzymes, we made predictions about which residues are functional significant to 2SL (including residue N13)




**5. Site-Directed Mutagenesis**
Using primers with a mismatch at position 13, we introduced a mutation converting polar asparagine to non-polar isoleucine (N13I). Then, we treated the PCR product with *Dpn1* to digest the methylated template DNA.

## RESULTS

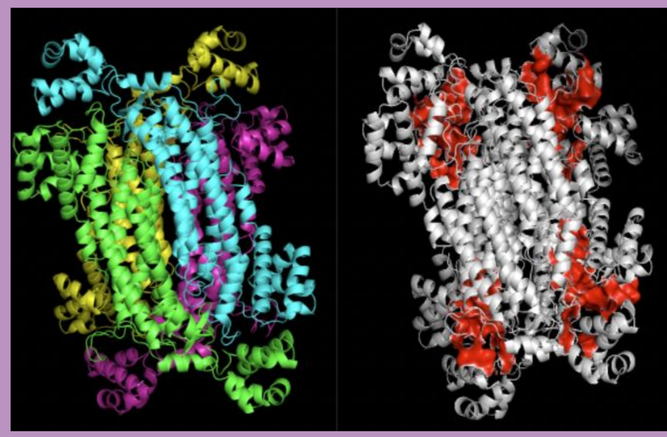
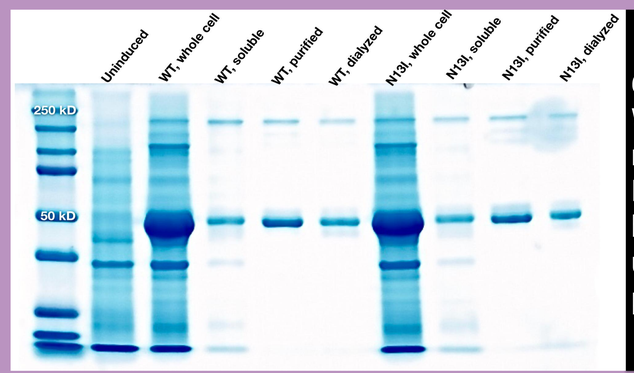


**Figure 2: The *Dickeya dadantii* 2SL model.** Sequences were run through https://swissmodel.expasy.org/. A model was produced using the known crystal structure of Fumarate lyase from *Mesorhizobium* (right). The homologous residues of active sites on three different lyase I-like enzymes were marked on the 2SL models (left).

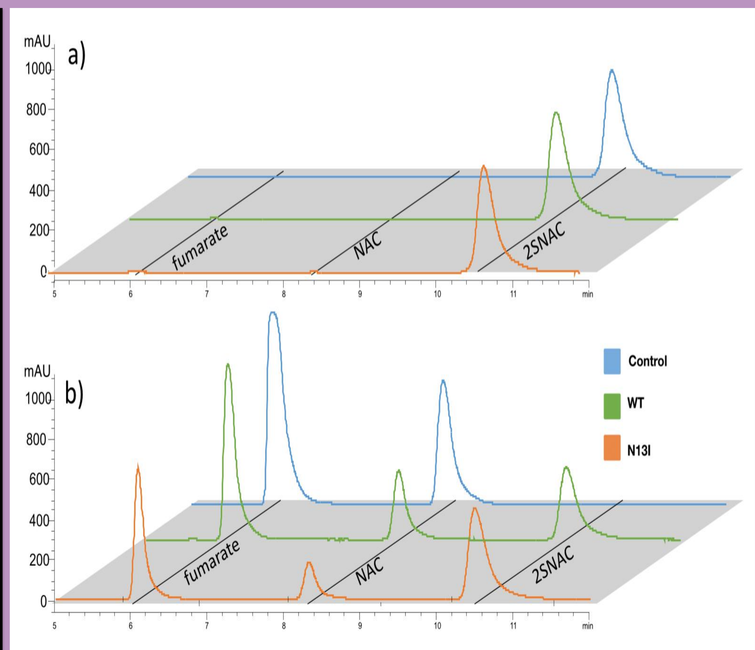



**6. SDS-PAGE gel of WT and mutant 2SL**
We were able to express and purify both mutant and WT 2SL N-terminal HIs-tagged proteins to near homogeneity. Proteins were purified using nickel chromatography. Target proteins are 52 kDa.

**7. HPLC Assays**
The reactions were run at 37℃ for one minute before they were stopped. Assays used a mobile phase of 0.1% TFA and 3% acetonitrile. The retention times are 6 minutes for fumarate, 8 for NAC, and 10 for 2SNAC.

a) The forward reaction assays produced peaks of heights 1.5 (fumarate), 1.7 (NAC), and 522.4 (2SNAC) for the WT enzyme. Peak heights for the N13I mutant were 9.4 (fumarate), 1.8 (NAC), and 532.1 (2SNAC).

b) The reverse reaction assays produced peaks of area 11197.5(fumarate), 4872.8(NAC), and **6998.4** (2SNAC) for the WT enzyme. Peak areas for the N13I mutant were 7127.3 (fumarate), 2583.4 (NAC), and **9251.5** (2SNAC).




## DISCUSSION

**The HPLC assay of the reverse reaction seems to suggest that the mutant N13I enzyme is more efficient (product peak area = 9251.5) than the WT (product peak area = 6998.4), at least in the reverse direction. This trend seemed to hold true through multiple trials of the assay.**

**The disparity between the product peaks in the forward reaction was smaller. Upon a second HPLC assay, the results of the forward reaction were not replicated.**

**Only one run of the HPLC assay is shown, more assays will need to be done in the forward direction to determine whether the results are conclusive.**

## FUTURE DIRECTIONS AND ACKNOWLEDGEMENTS

**Next steps will look like repeating the HPLC assays for the forward direction. Then, we will kinetically characterize the mutant enzyme. This will give us further experimental evidence that the N13 residue is functionally significant to 2SL activity. Finally, we will repeat the process with more mutants by altering different 2SL residues. This information could aid in a broader search for 2SL enzymes and 2SC breakdown pathways throughout other kingdoms. The identification of more 2SC catabolic pathways will contribute insight to the ongoing efforts to understand and treat diseases such as diabetes, obesity, and cancer.**

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